Universidade de Lisboa

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Effect of Chlorhexidine Incorporation on the Surface Properties of Acrylic Reline Resins

Marta Cristina Silvério Barreiros

Dissertação

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Dissertação orientada pela Professora Doutora Maria Cristina Bettencourt Neves e coorientada pela Professora Doutora Ana Francisca Bettencourt

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Resumo

A progressiva reabsorção do osso alveolar após perda dentária tem como consequência a desadaptação de próteses dentárias. Nestas situações, de forma a recuperar a retenção, a estabilidade e a distribuição equitativa de forças pelos tecidos de suporte, as próteses devem ser alvo de um rebasamento. Este procedimento é realizado com resinas acrílicas autopolimerizáveis em meio clínico (rebasamento direto) ou em meio laboratorial (rebasamento indireto). Apesar destas vantagens, estes materiais apresentam desvantagens, como o odor desagradável, alterações de coloração, porosidade e fácil degradabilidade, contribuindo para uma irritação química da mucosa oral e, consequentemente, para o aumento da suscetibilidade à colonização microbiana.

A estomatite protética é uma condição crónica que se manifesta por uma inflamação assintomática dos tecidos de suporte de próteses dentárias. É considerada a lesão oral mais comum, sendo observada em 60-70% dos pacientes que utilizam próteses. Apesar da sua etiologia multifatorial, muitos autores apoiam a associação entre espécies de *Candida*, especialmente *Candida albicans*, com o desenvolvimento de estomatite protética, já que estes microrganismos apresentam elevada capacidade de aderência ao polimetilmetacrilato, principal constituinte da resina acrílica das próteses dentárias.

Deste modo, a inibição da formação de biofilmes de *C. albicans* é particularmente importante na prevenção da estomatite protética. Os tratamentos disponíveis compreendem terapia antifúngica tópica ou sistémica, higiene oral meticulosa e restabelecimento nutricional.

A clorexidina é um agente antimicrobiano de largo espetro de ação contra microrganismos, incluindo *C. albicans*, sendo prescrito comummente em Medicina Dentária como solução de bochecho a 0,2%. O seu efeito antifúngico tem sido amplamente estudado por vários estudos, que demonstram uma supressão da capacidade de aderência de *C. albicans* a células epiteliais quando exposta a clorexidina. No entanto, a maioria do agente é removido da cavidade oral durante a primeira hora pela reposição salivar, minimizando as suas propriedades terapêuticas. Além disso, a resina acrílica da base da prótese pode atuar como um reservatório de microrganismos, contribuindo para a reinfeção da mucosa oral, que pode ocorrer até duas semanas após tratamento. Tem sido sugerido que a fraca penetração do fármaco no biofilme microbiano e a baixa adesão do paciente à terapêutica são as principais causas da recorrência de estomatite protética.

Deste modo, têm sido investigadas alternativas para o tratamento da estomatite protética, como sistemas de libertação de fármacos. A impregnação de resinas acrílicas com clorexidina assenta no pressuposto que esta se vai libertando a um ritmo constante, com um nível terapêutico baixo, inibindo a aderência e o crescimento de microrganismos, com riscos mínimos de toxicidade sistémica. Alguns estudos que avaliaram a libertação de clorexidina de resinas acrílicas concluíram que esta apresentava uma taxa inicial de eluição elevada, seguida de um processo de libertação controlado e estável.

A concentração de clorexidina mais utilizada nestes estudos é de 10% por massa de pó de resina acrílica, tendo sido demonstrado que é uma abordagem exequível e eficaz. No entanto, a literatura é escassa no que toca às consequências da incorporação de clorexidina nas propriedades dos materiais.

Assim sendo, o objetivo deste estudo foi avaliar o efeito da incorporação de clorexidina na resistência ao corte de duas resinas de rebasamento direto, Kooliner e Ufi Gel Hard, e de uma resina acrílica de rebasamento indireto, Probase Cold, quando aderidas a uma resina termopolimerizável para base da prótese (Probase Hot). Foi também avaliada a influência da incorporação de clorexidina na energia de superfície das mesmas três resinas acrílicas de rebasamento.

Para o teste de resistência ao corte, foram preparados cento e oitenta espécimes de resina para base da prótese (12×10×6 mm). Após polimerização, os espécimes foram submetidos a 2 500 ciclos de termociclagem (5-55 ºC). Todos os espécimes foram reduzidos a 3 mm de espessura e divididos em três grupos, correspondentes a cada uma das resinas acrílicas, sendo, por sua vez, divididos em 6 grupos (n=10). As resinas de rebasamento foram unidas à superfície preparada da resina para base da prótese. Às amostras experimentais incorporou-se clorexidina em pó em proporções de 1%, 2,5%, 5%, 7,5% ou 10% da massa do pó da resina acrílica, consoante o grupo de estudo. Depois do armazenamento em água destilada numa estufa a 37 ºC durante 24h, os espécimes foram submetidos a testes mecânicos de resistência ao corte, com uma máquina de testes universal Instron, utilizando uma velocidade de 1 mm/min. As superfícies foram observadas ao estereomicroscópio para determinar o tipo de falha, sendo estas classificadas como: adesiva, se não existiam vestígios de resina de rebasamento na resina de base da prótese ou vice-versa; mista, se existiam vestígios de resina de rebasamento na resina de base da prótese ou vice-versa; coesiva, se toda a superfície da base da prótese estava preenchida por resina de rebasamento.

Para avaliação da energia de superfície das diferentes resinas acrílicas de rebasamento, estas foram colocadas no interior de moldes de aço (125×25×1 mm). Às amostras experimentais foi incorporada clorexidina nas mesmas proporções descritas anteriormente, consoante o grupo de estudo. Após polimerização, foram seccionadas de forma a obter 30 espécimes por resina acrílica, divididas em 5 grupos de estudo e um grupo controlo (*n*=5). Através da técnica da Placa de *Wilhelmy*, no tensiómetro de *Kruss*, os ângulos de contacto foram determinados e a energia de superfície foi posteriormente calculada pelo método de *Wu*.

Os resultados foram analisados estatisticamente através de testes não paramétricos, pelo método de *Kruskal-Wallis*, seguindo-se múltiplas comparações pelos testes de *Mann-Whitney*, com correção de *Bonferroni*. Em todos os testes estatísticos foi considerado o nível de significância de 5%.

Foram encontradas diferenças estatisticamente significativas nos valores dos testes de resistência ao corte, entre as resinas de rebasamento, sendo que os espécimes de Kooliner demonstraram valores significativamente inferiores aos valores das outras duas resinas. Os espécimes de Kooliner e Ufi Gel Hard não demonstraram diferenças significativas entre grupos experimentais. No entanto, os espécimes de Probase Cold obtiveram uma diminuição dos valores de resistência ao corte proporcional à concentração de clorexidina incorporada.

Após análise das superfícies de todos os espécimes com estereomicroscópio, obtiveram-se: 98,33% falhas adesivas no grupo Kooliner; 38,33% falhas adesivas e 61,67% falhas mistas no grupo Ufi Gel Hard; e 51,67% falhas adesivas e 48,33% falhas mistas no grupo Probase Cold.

Em relação à energia de superfície, foram encontradas diferenças significativas entre os materiais nos valores de energia de superfície total e do componente dispersivo, sendo que o Ufi Gel Hard demonstrou valores significativamente mais elevados de energia de superfície total e de componente dispersivo do que as outras resinas. Os valores de componente polar não revelaram diferenças significativas entre resinas acrílicas.

Os espécimes de Kooliner mostraram diferenças significativas entre grupos nos valores de energia de superfície total, de componente dispersivo e de componente polar.

Diferenças significativas foram encontradas entre grupos de Ufi Gel Hard e de Probase Cold nos valores de energia de superfície total. Os componentes dispersivo e polar não obtiveram diferenças significativas nas duas resinas.

Em suma, a incorporação de clorexidina afeta a resistência ao corte apenas nos espécimes de Probase Cold com concentração elevada de clorexidina, já que a mesma não mostrou diferenças significativas nos espécimes de Kooliner, Ufi Gel Hard e Probase Cold até uma concentração de clorexidina de 2,5%. A energia e superfície das resinas de rebasamento em estudo mostra diferenças com a incorporação de clorexidina em todas as resinas: Ufi Gel Hard e Probase Cold demonstraram uma tendência para adquirir maior polaridade com a incorporação de clorexidina, enquanto que os resultados obtidos com Kooliner não permitiram estabelecer uma relação com as propriedades de superfície.

Tendo em vista um conhecimento mais aprofundado da influência da incorporação de clorexidina em resinas acrílicas, outros estudos deveriam ser realizados, recorrendo a outros métodos de teste das propriedades de adesão. Além disso, o efeito da energia de superfície na adesão de *C. albicans* ainda permanece por explicar, pelo que são necessários estudos microbiológicos. Seria, igualmente útil a realização de testes à rugosidade de superfície, uma vez que esta propriedade está relacionada com a colonização da superfície das resinas acrílicas por microrganismos.

Palavras-chave: Incorporação de fármacos, Clorexidina, Resistência ao corte, Energia de superfície, Resinas acrílicas.

Abstract

A release delivery system for the treatment of denture stomatitis using chlorhexidine-incorporated self-cured resins has been investigated.

The main purpose of this study was to evaluate the effect of chlorhexidine incorporation on shear bond strength and surface free energy of three acrylic reline resins, Kooliner, Ufi Gel Hard and Probase Cold.

For all tests, the experimental specimens were incorporated with chlorhexidine 1%, 2.5%, 5%, 7.5% or 10% (w/w) and the control specimens were left unloaded.

Shear bond strength test was performed on specimens of reline resins attached to denture base resin (n=10) and after this test, the failure mode was assessed.

Specimens with 25×16×1 mm dimensions (n=5) were submitted to contact angles determination, performed by the Wilhelmy plaque technique, in order to estimate surface free energy values.

Statistical differences were observed among acrylic reline resins in shear bond strength values, with Kooliner showing significantly lower values than the other reline resins. Statistical differences weren't found between groups from Kooliner and Ufi Gel Hard. Probase Cold specimens obtained a decrease of shear bond strength values with the increase of chlorhexidine concentration incorporated.

Statistical differences were observed among acrylic reline resins in total surface free energy values, with Ufi Gel Hard demonstrating total surface free energy values significantly higher than the other reline resins, at cost of the increased values of dispersive component. Statistical differences were found between all groups in total surface free energy values from Kooliner, Ufi Gel Hard and Probase Cold. However, only Kooliner showed significant differences in dispersive and polar components.

In sum, the incorporation of chlorhexidine has influence only on shear bond strength of Probase Cold specimens with higher concentrations of chlorhexidine. The surface free energy of the three acrylic reline resins is affected by the incorporation of chlorhexidine.

Keywords: Drug incorporation, Chlorhexidine, Shear bond strength, Surface free energy, Acrylic resins.

1. Introduction

In the last decade, the adult population has been experiencing an improvement in oral health, leading to a decrease of edentulism. However, the demographic trends of an increase in size and age of the older population leads to a still significant number of patients needing treatment that require dentures (Douglass, *et al.*, 2002; Barbosa, *et al.*, 2008).

Tooth loss results in a progressive alveolar bone resorption that may contribute to a less stable denture (Leles, *et al.*, 2001). In these situations, prosthesis may be relined, improving its retention, stability and the masticatory load distribution that is transmitted to the underlying tissues (Aydın, *et al.*, 1999; da Silva, *et al.*, 2014). The relining procedure can be carried out with autopolymerizing acrylic resins at the chairside in the dental clinic or as a laboratory procedure (Leles, *et al.*, 2001; Neppelenbroek, *et al.*, 2006). However, these materials have some disadvantages, such as unpleasant odour, colour alteration, porosity and easy degradability, which contributes to chemical irritation of the oral mucosa and a higher susceptibility to microbial colonization, and consequently denture stomatitis (Leles, *et al.*, 2001; Pinto, *et al.*, 2004; Elias and Henriques, 2007; Bettencourt, *et al.*, 2010; Alcântara, *et al.*, 2012).

Denture stomatitis is a chronic condition that manifests as a diffuse usually asymptomatic inflammation of the denture-bearing areas and it is considered the most common oral lesion, observed in 60-70% of denture wearers (Webb, *et al.*, 1998; Redding, *et al.*, 2009; Cao, *et al.*, 2010; da Silva, *et al.*, 2011; Ryalat, *et al.*, 2011; Hahnel, *et al.*, 2012; Koch, *et al.*, 2013). The etiology of this condition has been suggested as multifactorial, including trauma from ill-fitting dentures, presence of biofilm, alteration in salivary pH, poor hygiene, continuous denture wear, nutritional deficiency, antibiotic drug use, immune suppression and xerostomia (de Sousa, *et al.*, 2009; Redding, *et al.*, 2009; Alcântara, *et al.*, 2012; AL-Dwairi, *et al.*, 2012). Beside these contributing factors, several studies support the strong association of *Candida* species, especially *C. albicans*, with the development of denture stomatitis, since these microorganisms have the ability to adhere to polymethylmethacrylate (PMMA), which constitutes the acrylic resin (Pinto, *et al.*, 2004; de Sousa, *et al.*, 2009; Redding, *et al.*, 2009; da Silva, *et al.*, 2011; Salim, *et al.*, 2012b; Bertolini, *et al.*, 2014).

Inhibition of the formation of *C. albicans* biofilms on protheses may be very important in preventing the development of denture stomatitis (Redding, *et al.*, 2009). The treatments available include topical or systemic antifungal therapy, careful oral and denture hygiene and nutritional restitution (Amin, *et al.*, 2009; Alcântara, *et al.*, 2012).

Chlorhexidine (CHX) is an antimicrobial agent widely prescribed as an antiseptic mouthwash in dentistry due to its activity against a wide range of microorganisms, including *Candida* (Amin, *et al.*, 2009; Ryalat, *et al.*, 2011; Salim, *et al.*, 2013a). The antifungal effect of CHX has been presented in several studies, and has been demonstrated that exposure of *C. albicans* to CHX suppresses its ability to adhere to buccal epithelial cells (Redding, *et al.*, 2009; Ryalat, *et al.*, 2011; Salim, *et al.*, 2013a). In addition, CHX has been showing low concentration efficiency, substantivity, capacity to reduce biofilm formation and disorganize pre-formed biofilm (de Sousa, *et al.*, 2009).

There are many oral delivery systems for CHX, being a 0.2% mouthwash the most used mode. However, the majority of the agent is removed from the oral cavity during the first hour due to the diluent and cleansing effects of saliva, minimizing its therapeutic properties (Ryalat, *et al.*, 2011). Furthermore, acrylic resin denture bases may act as a reservoir for microorganisms and thus contribute to re-infection of the treated oral mucosa, which may occur up to two weeks post-treatment. It has been suggested that poor drug penetration into the microbial film on the denture material and poor patient compliance, due to the need for frequent drug application or associated adverse effects (such as mild disconfort, epithelium exfoliation and teeth staining) may be the main causes to denture stomatitis recurrence (de Sousa, *et al.*, 2009; Ryalat, *et al.*, 2011; Alcântara, *et al.*, 2012; AL-Dwairi, *et al.*, 2012; Salim, *et al.*, 2012b).

A release delivery system for the treatment of denture stomatitis using CHX-incorporated self-cured resins has been investigated. In several studies, the general principle is to impregnate dentures with CHX that releases from the device and inhibits microbial adherence and growth (Cao, *et al.*, 2010; Salim, *et al.*, 2013a; Bertolini, *et al.*, 2014). A topical sustained release dosage form helps saturate the salivary film, which bathe the surface of a denture base with a continuous release of antimicrobial drugs at a low therapeutic level, with minimal risk of systemic toxicity and over a long period of time (Ryalat, *et al.*, 2011; Alcântara, *et al.*, 2012; Salim, *et al.*, 2012a; Salim, *et al.*, 2013a; Bertolini, *et al.*, 2014). By incorporating antimicrobial agents into resin-based denture relining materials, it is possible not only to create a drug delivery system, but also overcome the side effects of mouth rinsing with CHX and guarantee availability of the

agent in the target area at a therapeutic dosage (Amin, *et al.*, 2009; Ryalat, *et al.*, 2011; Salim, *et al.*, 2013b; Bertolini, *et al.*, 2014). Hence, the duration of the therapy is extended, the monitoring is minimal and the patient compliance is not required (Salim, *et al.*, 2012a; Salim, *et al.*, 2013a).

Some studies have evaluated the CHX release from acrylic resins and concluded that there is a high initial rate of delivery from the material, followed by a controlled slow and steady diffusion (Hiraishi, *et al.*, 2008; Amin, *et al.*, 2009; Ryalat, *et al.*, 2011; Salim, *et al.*, 2013b).

The CHX concentration that has been most used in earlier studies that evaluate the efficacy of a release delivery system against *C. albicans* is 10% (w/w) and this treatment has shown to be effective and feasible (Amin, *et al.*, 2009; Ryalat, *et al.*, 2011; Salim, *et al.*, 2012b; Salim, *et al.*, 2013b). However, the incorporation of drugs into polymeric materials may affect their mechanical properties, as flexural strength, and surface properties, as hardness, surface free energy and bond strength of the reline material to the denture base resin (Addy and Handley, 1981; Alcântara, *et al.*, 2012; Salim, *et al.*, 2012a).

An adequate bonding between the denture base resin and the reline resin is essential as it directly affects the clinical performance of the components (Giampaolo, *et al.*, 2011; Alcântara, *et al.*, 2012). A weak bond encourages the ingress of oral fluids and microorganisms at their junction, leading to debonding of the two materials and staining (Leles, *et al.*, 2001; Takahashi and Chai, 2001b; Pinto, *et al.*, 2004; Mutluay and Ruyter, 2005; Bettencourt, *et al.*, 2010; Giampaolo, *et al.*, 2011). Moreover, a strong bond is also critical for safe, durable and effective delivery of the drug (Salim, *et al.*, 2012a).

Surface free energy is an important parameter for the understanding of the biological performance of biomaterial surfaces, in particular, for cellular adhesion (Shabalovskaya, *et al.*, 2013). Some studies have demonstrated a linear relationship between surface free energy values and *C. albicans* adherence, where the higher the surface free energy, i. e. the more hydrophobic the surface, the higher will be the adhesion of microorganisms. Thus, surface energy may contribute to the adherence, bonding and colonization of *Candida* species (AL-Dwairi, *et al.*, 2012).

Despite its importance, scarce literature was found showing the impact of CHX incorporation with different concentrations on the surface properties of acrylic reline resins.

2. Objectives

The main purpose of this work was to evaluate the effect of different concentrations of CHX incorporation on the shear bond strength of three acrylic reline resins to one heat-polymerizing base resin and on the surface free energy of the reline resins, according to the following hypotheses:

H0: The acrylic reline resin used doesn't affect the shear bond strength to denture base resin.

H1: The acrylic reline resin used affects the shear bond strength to denture base resin.

H0: The shear bond strength isn't affected by different concentrations of CHX incorporated.

H1: The shear bond strength is affected by different concentrations of CHX incorporated.

H0: The surface free energy values aren't different between acrylic reline resins.

H1: The surface free energy values are different between acrylic reline resins.

H0: The values of surface free energy don't differ among different concentrations of CHX incorporated.

H1: The values of surface free energy differ among different concentrations of CHX incorporated.

3. Materials and Methods

This study aimed to assess if the incorporation of different concentrations of CHX would affect the surface free energy and bond strength between three reline resins and a denture base resin, as well as if there were differences among the reline resins.

The materials assessed in this study (Appendix 2, Figure 1, 2, 3 and 4) include one heat-polymerizing denture base acrylic resin, Probase Hot (Ivoclar Vivadent AG, Liechtenstein) and three autopolymerizing acrylic reline resins, Kooliner (GC America Inc, Alsip, Illinois, USA), Ufi Gel Hard (Voco GmbH, Cuxhaven, Germany) and Probase Cold (Ivoclar Vivadent AG, Liechenstein). These three auto-polymerizing acrylic resins were selected for they differ in chemical composition. The name, manufacturer, composition, power/liquid ratio, polymerization condition and batch number of the materials used in this investigation are listed in Table 3.1.

Table 3.1 – Materials used in the study.

Product	Manufacturer	Composition		Powder/Liquid Ratio (g/mL)	Polymerization Condition	Batch Number
		Powder	Liquid			
Probase Hot (PH)	Ivoclar Vivadent AG, Liechtenstein	PMMA	MMA	22.5 / 10	Heat-polymerization Heat up to 100 ºC and let boil for 45 minutes	S14737 (P) S25951 (L) N31963 (SF)
Kooliner (K)	GC America Inc., Alsip, Illinois, USA	PEMA	IBMA	1.4 / 1	Autopolymerization 10 minutes at room temperature	1406232 (P) 1404241 (L)
Ufi Gel Hard (U)	Voco GmbH, Cuxhaven, Germany	PEMA	1,6-HDMA	1.77 / 1	Autopolymerization 7 minutes at room temperature	1438417 (P) 1443063 (L) 1432118 (CON)
Probase Cold (PC)	Ivoclar Vivadent AG, Liechtenstein	PMMA	MMA	1.5 / 1	Autopolymerization 15 minutes at 40 ºC 2-4 bar	S41038 (P) U03356 (L)

P = Powder, L = Liquid, SF = Separating Fluid, CON = Conditioner, PMMA = Polymethylmethacrylate, MMA = Methylmethacrylate, PEMA = Polyethylmethacrylate, IBMA = Isobutylmethacrylate, HDMA = Hexanedioldimethacrylate.

3.1. Shear bond strength

Preparation of denture base specimens

One hundred and eighty denture base specimens were prepared using a modified flasking technique. A silicon mold was used to obtain rectangular wax specimens (12×10×6 mm), which were then flasked and placed on top of the investment with gypsum type III. The first layer of gypsum was coated with vaseline and in the upper half of the flask an investment of gypsum type IV was placed, covering the specimens. The flask lid was put in place before the second layer set, allowing the excess gypsum to flow out of the holes. After the complete set of the gypsum, the flask was placed in boiling water for 4 to 6 minutes. Afterwards, it was removed from the water, opened and the wax was removed. A separating fluid (Ivoclar Vivadent AG, Liechtenstein) was applied on the gypsum and the heat-polymerizing resin was manipulated and packed into the flask. The flask was then pressed using a hydraulic system and polymerized according to manufacturer's instructions (Table 3.1). The flasks were removed from the water bath and cooled to room temperature before specimens were removed.

After the 180 specimens were removed from the molds, their sides were grounded in a rotational grinding and polishing machine (DAP-U, Struers, Denmark) with 600-grit silicon carbide paper (Carbimet Paper Discs, Buehler Ltd., Lake Bluff, IL) under water supply to remove irregularities.

All specimens were treated by a standardized thermocycling aging procedure of 2 500 cycles with thermal fluctuations between 5 ºC and 55 ºC (20 seconds each bath) and 5 seconds of dwell time in a specific machine (Refri 200-E, Aralab, Cascais, Portugal) (Appendix 2, Figure 6).

Relining procedure

Surfaces of denture base specimens were reduced to a 3 mm thickness using a rotational grinding and polishing machine (DAP-U, Struers, Denmark) with 600-grit silicon carbide paper (Carbimet Paper Discs, Buehler Ltd., Lake Bluff, IL), which simulates the preparation of the denture base to be relined. The thickness was confirmed with digital micrometer (Mitutoyo Digimatic, MFG.Co., Ltd. Tokyo, Japan) with precision ± 0.01 mm.

The 180 denture base specimens were randomly divided into three groups, corresponding to the three different acrylic reline resins. The bonding area of 3 mm in diameter was customized and defined with a perforated adhesive tape (Glossy White Film EA, Xerox), placed on the center of the surface of denture base providing a uniform bonding area (Figure 3.1). The denture base specimens were then stabilized with gypsum type III.

The bonding sites of Kooliner and Probase Cold specimens were wetted with the corresponding monomer. To the Ufi Gel Hard specimens, it was applied a specific adhesive on its surface and let it dry in the air (30 seconds), as recommended by the manufacturer.

The 60 specimens corresponding to each acrylic reline resin were randomly divided into six groups (n=10). On experimental specimens, the acrylic reline resin was incorporated with chlorhexidine diacetate monohydrate (Panreac Applichem, Darmstadt, Germany) (CHX) (Appendix 2, Figure 5) at a proportion of 1%, 2.5%, 5%, 7.5% and 10% of the acrylic resin's powder weight (w/w). A mortar and pestle was used to homogenize the mixture (Figure 3.2). Each acrylic reline resin was mixed and applied according to the manufacturer's instructions (Table 3.1).

With direct relining materials, polymerization was carried out at 37 ºC to simulate the temperature of the oral cavity during the specific time recommended by the manufacturer (Table 3.1). For the indirect relining material, an Ivomat pressure device (IvoclarVivadent, Lichenstein) was used to maintain 40 ºC and 2-4 bar for 15 minutes (Table 3.1) (Appendix 2, Figure 7).

All specimens were then stored in distilled water at 37±2 ºC for 24±2 hours in an incubator (Memmert, Schwabach, Germany) before shear bond strength tests.

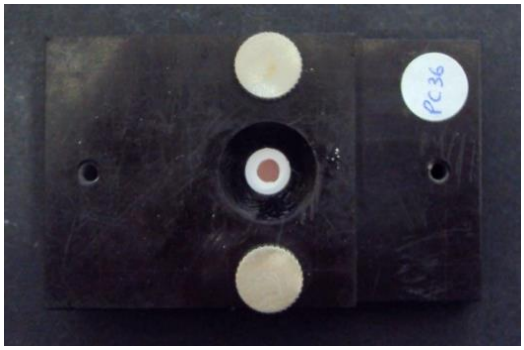

Figure 3.1 – Shear bond strength device.

Figure 3.2 - Incorporation and homogenization of CHX into the acrylic resin's powder.

Shear bond strength test

Specimens were included in a single plan lap shear bond strength device (Figure 3.3) with gypsum type III and tested in an universal testing machine model 4502 (Instron Ltd, Bucks, HP 12 3SY, England) (Figure 3.4a). Shear bond strength was determined with 1kN load cell and a crosshead speed of 1 mm/min until debonding of the materials (Figure 3.4b). All tests were performed under uniform atmospheric conditions at room temperature.

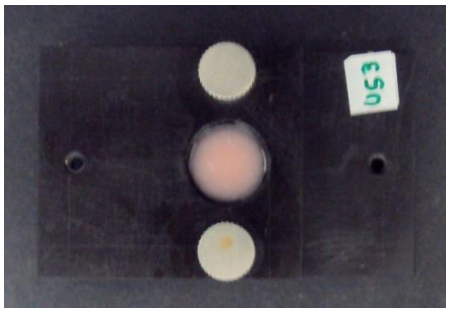

Figure 3.3 - Shear bond strength device filled with Ufi Gel Hard.

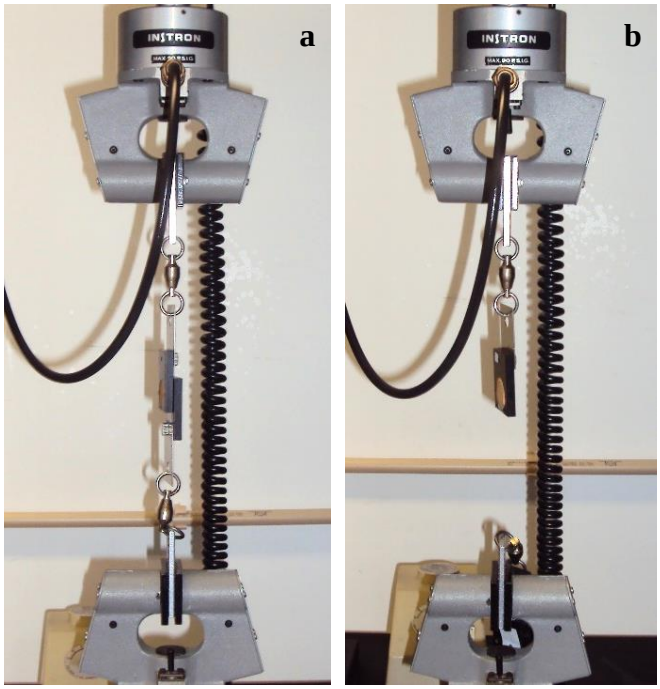

Figure 3.4 – One example of specimen submitted to shear bond strength test in an universal testing machine.
a) Before test; b) After test.

Failure Mode

After shear bond testing (Figure 3.5), the detached surfaces of specimens were examined with a stereomicroscope (EMZ-8TR, Meiji Techno Co, Saitama, Japan). The failure mode was classified by 2 independent observers as adhesive, mixed or cohesive. Failures that occurred at the reline base resin interface, leaving no trace of any reline material on the denture base or vice versa, were registered as adhesive failure. If it was detected any trace of denture polymer on the surface of the denture reline polymer or vice versa, the failure was classified as mixed. When the entire surface of the denture base was filled with denture reline polymer, the failure mode was recorded as cohesive.

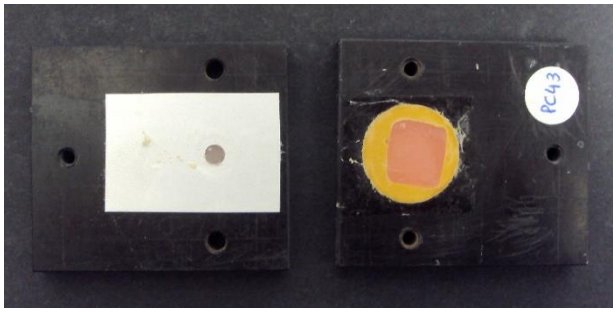

Figure 3.5 – Shear bond strength device after submitted to shear bond strength test.

3.2. Surface free energy

Preparation of specimens

Specimens were prepared by packing the mixture from each acrylic reline resin (Kooliner, Ufi Gel Hard and Probase Cold) into rectangular metal molds (125×25×1 mm), according to manufacturer's instructions (Table 3.1). On experimental specimens, the acrylic reline resin was incorporated with chlorhexidine diacetate monohydrate (Panreac Applichem, Darmstadt, Germany) (CHX) (Appendix 2, Figure 5) at a proportion of 1%, 2.5%, 5%, 7.5% and 10% of the acrylic resin's powder weight (w/w). A mortar and pestle was used to homogenize the mixture (Figure 3.2). Each mold was clamped together in order to displace any material's excess (Figure 3.6). After polymerization with specific conditions according to the manufacturer's instructions (Table 3.1) (Figure 3.7), the samples were removed from the molds and were cut into rectangular specimens with approximate dimensions of 25mm width, 16mm height and 1mm thickness, obtained from the cured strips. The edges of each sample were polished manually with a 600-grit silicon carbide paper (Carbimet Paper Discs, Buehler Ltd., Lake Bluff, IL) in order to remove any irregularities.

Thirty specimens of each material were obtained, divided by six groups of five samples (n=5) according to different concentrations w/w of CHX (0%, 1%, 2.5%, 5%, 7.5% and 10%).

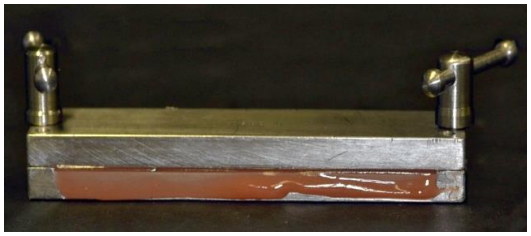

Figure 3.6 – Compression of one resin's dough in the metal mold.

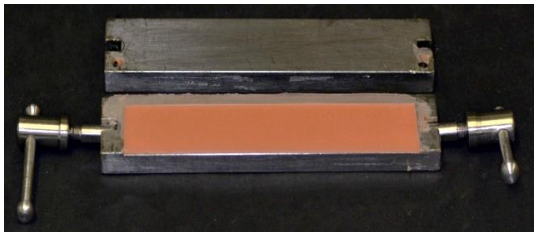

Figure 3.7 – Metal mold opened after polymerization of acrylic reline resin.

Surface free energy determination

The determination of the acrylic reline resin specimen's surface free energy is estimated by measuring contact angles of distilled water and 1,2-propanediol on 5 specimens from each group, using Wilhelmy plate technique (Bettencourt, *et al.*, 2004). Testing was carried out using a Processor Tensiometer K12 (Kruss, Hamburg, Germany) linked to a computer and the advancing and regression contact angle were measured (Appendix 2, Figures 8, 9, 10 and 11).

The system was set in a "Perspex®" box to ensure an artificially controlled environment. A glass cuvette containing the liquid was placed in a steel container with thermostatic circulating water (25±1 ºC). Before each change of the liquid, the cuvette glass was carefully washed with a water and acetone mixture and was further assed into the flame of a Bunsen burner to reduce the likelihood of surface contamination.

Primarily, the specimen's dimensions (height, width and thickness) were measured with digital micrometer (Mitutoyo Digimatic, MFG.Co., Ltd Tokyo, Japan) with precision ± 0.01 mm and introduced in the software. At the beginning of each experiment, specimen of acrylic reline resin was suspended in the balance (sensitivity equal to 10^{-4} g) of the equipment. A motorized platform allowed the immersion of 4 mm of specimen in the liquid under study (water or 1,2-propanediol) at a speed of 20 μm s^{-1} (Figures 3.8 and 3.9). In all the procedure, care was taken not to handle the surfaces of the specimens to reduce the chance of contamination.

Advancing contact angles were used for surface free energy (γ) estimation of all specimens, as well as its dispersive (γ^d) and polar components (γ^p) based on the harmonic mean method proposed by Wu (1971). Equations for surface free energy estimation were solved using the equation handling KRUSS-software program: contact angle measuring system K121 (version 2.049) (Appendix 2, Figure 12).

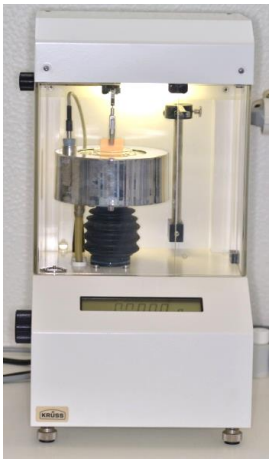

Figure 3.8 – Specimen of acrylic reline resin suspended in the balance of the equipment.

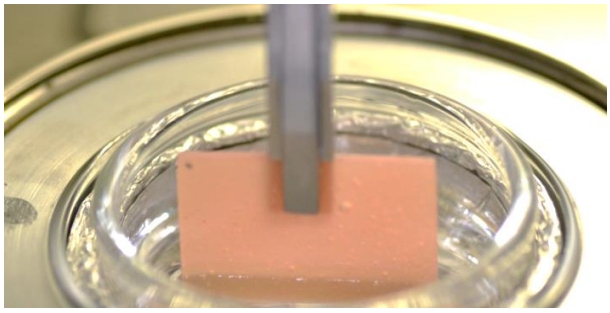

Figure 3.9 – Specimen of acrylic reline resin immersed in the glass cuvette with distilled water.

3.3. Statistical analysis

Data were statistically analyzed using SPSS Statistics 20 (SPSS Inc., Chicago, IL, USA). Since data did not follow a normal distribution for the studied variables (verified by Kolmogorov-Smirnov normality tests), the results were submitted to the nonparametric tests according to the Kruskal-Wallis method followed by multiple comparisons using Mann-Whitney tests with Bonferroni correction to determine whether there were specific significant differences among materials and concentrations.

In all statistical tests, it was considered the 5% level of significance ($p<0.05$).

4. Results

4.1. Shear bond strength

The results of the shear bond strength test are summarized in Table 4.1, where the mean, standard deviation, minimum and maximum values of each group were registered.

Table 4.1 – Shear bond strength data by reline resin.

Material	CHX Incorporation	*n*	Shear Bond Strength (MPa)		
			M±SD	Min	Max
Kooliner	**Control - 0%**	10	13.14±3.44	7.66	20.31
	1%	10	16.14±4.56	8.93	22.73
	2.5%	10	18.79±4.40	14.63	29.41
	5%	10	17.51±3.88	12.84	23.16
	7.5%	10	17.63±3.34	11.36	23.45
	10%	10	17.53±2.51	13.69	22.58
		60	16.38±1.72		
Ufi Gel Hard	**Control - 0%**	10	24.51±1.94	21.47	28.41
	1%	10	23.54±3.03	18.12	27.55
	2.5%	10	27.69±3.11	22.04	32.38
	5%	10	28.10±2.46	22.91	31.13
	7.5%	10	26.26±2.86	22.33	29.83
	10%	10	24.42±4.00	16.84	30.52
		60	25.75±1.88		
Probase Cold	**Control - 0%**	10	40.21±2.52	33.81	43.15
	1%	10	35.59±3.09	30.74	40.25
	2.5%	10	29.53±2.38	26.66	32.31
	5%	10	23.54±3.90	15.75	26.71
	7.5%	10	16.03±2.68	11.70	19.61
	10%	10	15.41±3.19	9.06	18.21
		60	26.72±10.21		

M = Mean, SD = Standard deviation, Min = Minimum, Max = Maximum

Statistical differences (p<0.001) were observed among acrylic reline resins in shear bond strength values (Figure 4.1). No statistical differences (p=1.000) were detected between Ufi Gel Hard and Probase Cold specimens, however Kooliner showed significantly lower (p<0.001) shear bond strength than the two other reline resins.

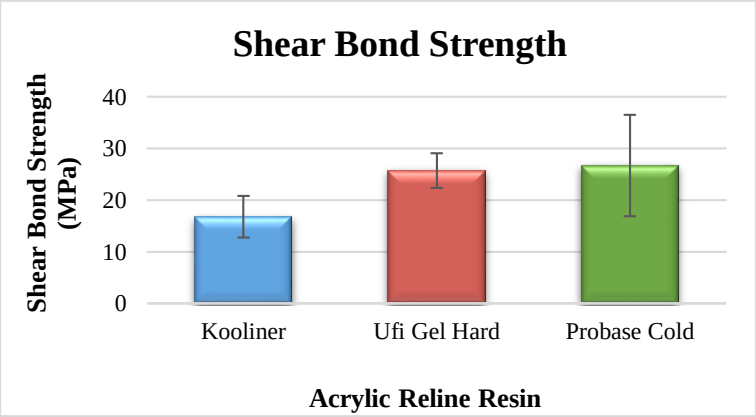

Figure 4.1 – Mean and standard deviation of values of shear bond strength (MPa) by acrylic reline resin.

For Kooliner specimens (Figure 4.2), no statistical differences (p=0.051) were found between groups.

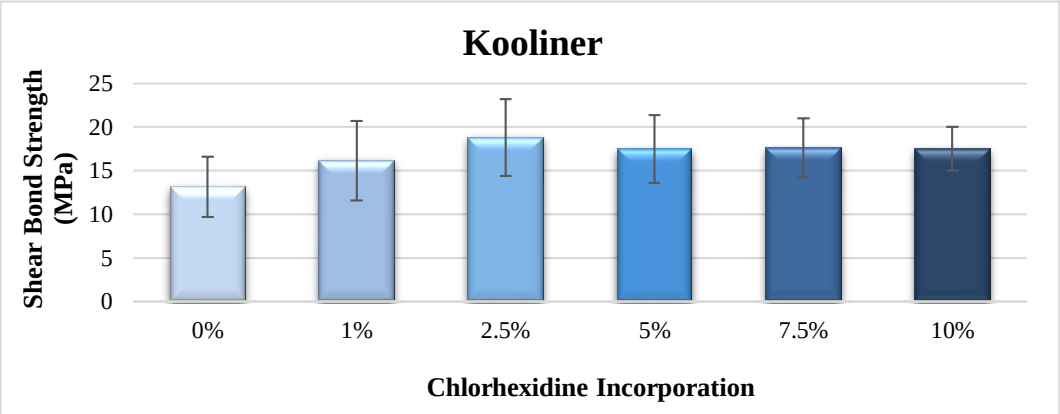

Figure 4.2 – Mean and standard deviation of shear bond strength values (MPa) of experimental groups of Kooliner.

For Ufi Gel Hard specimens (Figure 4.3), no statistical differences (p=0.05) were also establish between groups.

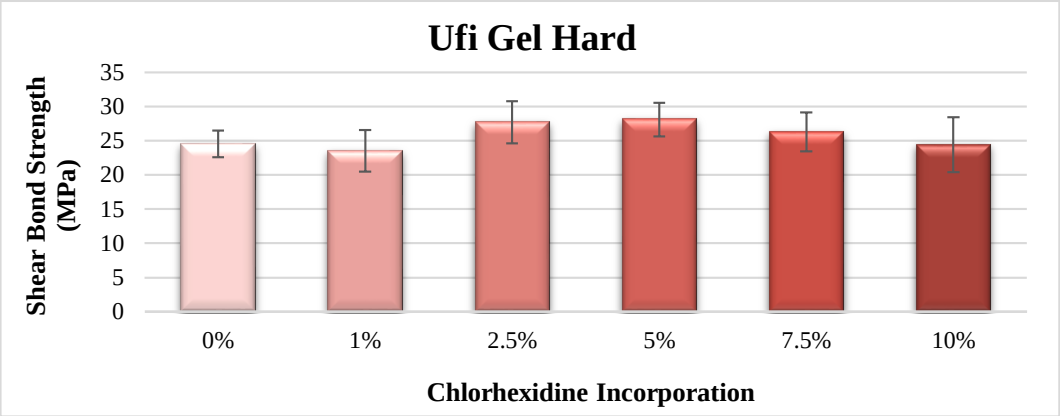

Figure 4.3 – Mean and standard deviation of shear bond strength values (MPa) of experimental groups of Ufi Gel Hard.

Statistical differences (p=0.001) were obtained among groups of Probase Cold specimens (Figure 4.4). 5% CHX Group showed bond strength values significantly lower than Control Group (p=0.001). 7.5% CHX Group and 10% CHX Group presented bond strength values significantly lower than Control Group (p<0.001), 1% CHX Group (p<0.001) and 2.5% CHX Group (p=0.026 and p=0.021, respectively). Among the other groups there were no statistical differences.

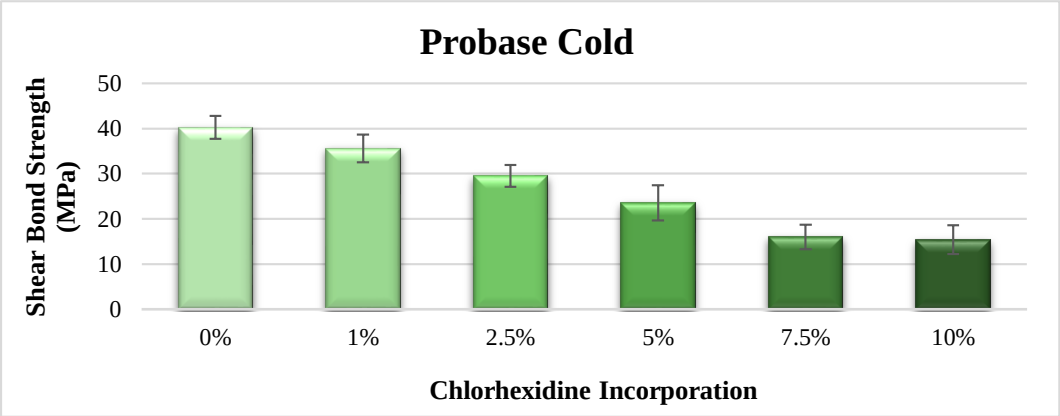

Figure 4.4 – Mean and standard deviation of shear bond strength values (MPa) of experimental groups of Probase Cold.

After analyzing all specimens' surfaces with a stereomicroscope (EMZ-8TR, Meiji Techno Co, Saitama, Japan), Kooliner specimens showed 98.33% of adhesive failures and 1.67% of mixed failures. On the other hand, Ufi Gel Hard obtained 38.33% and 61.67% of adhesive and mixed failures, respectively. Probase Cold presented a more even result, as 51.67% were adhesive failures and 48.33% were mixed failures (Appendix 6, Tables 1.1, 1.2 and 1.3).

4.2. Surface free energy

The descriptive analysis of the data was executed for each material, including mean, standard deviation, minimum and maximum values for contact angle (Appendix 1, Table 1) and surface free energy (γ) and their components, the dispersive (γ^d) and polar (γ^p) components (Appendix 1, Table 2).

The values of the total surface free energy and their components are summarized in Table 4.2, where the mean and standard deviation values of the groups by reline resin are listed.

Table 4.2 – Mean and standard deviation (M±SD) values for surface free energy by reline resin.

Material	CHX Incorporated	n	Surface Free Energy (γ) (mN/m)		
			γ Total	γ Dispersive	γ Polar
Kooliner	**Control - 0%**	5	24.48±1.82	14.48±5.85	10.06±6.56
	1%	5	25.68±0.91	16.60±2.04	9.08±1.67
	2.5%	5	27.92±3.83	19.64±1.16	10.26±1.90
	5%	5	33.04±1.10	21.06±1.30	12.00±1.44
	7.5%	5	31.56±1.34	14.50±7.87	17.06±7.77
	10%	5	30.40±1.50	16.88±1.86	13.52±3.13
		25	28.85±3.39	17.19±2.68	12.00±2.94
Ufi Gel Hard	**Control - 0%**	5	32.38±1.69	22.84±1.54	9.54±2.12
	1%	5	34.04±0.65	23.58±1.34	10.44±1.61
	2.5%	5	33.10±0.58	21.64±1.23	11.48±1.54
	5%	5	36.24±1.73	22.22±2.26	13.98±3.83
	7.5%	5	36.38±1.18	24.46±1.81	11.92±2.55
	10%	5	35.68±1.33	23.30±1.78	12.38±3.01
		25	34.64±1.70	23.01±1.00	11.62±1.55
Probase Cold	**Control - 0%**	5	24.62±1.79	14.60±1.21	10.02±2.54
	1%	5	25.94±1.67	14.60±1.24	11.34±2.49
	2.5%	5	28.78±1.72	15.98±1.72	12.78±2.99
	5%	5	30.84±1.20	15.96±1.30	14.88±2.18
	7.5%	5	26.40±2.08	14.24±1.61	12.18±0.89
	10%	5	29.32±1.39	15.18±1.41	14.14±2.71
		25	27.65±2.36	15.09±0.74	12.56±1.79

Statistical differences (p<0.001) were observed among acrylic reline resins in total surface free energy values (Figure 4.5). No statistical differences (p=0.695) were detected between Kooliner and Probase Cold specimens, but Ufi Gel Hard demonstrated total surface free energy values significantly higher (p<0.001) than the two other reline resins. Regarding the values of the disperse component, significant differences (p<0.001) were found between the three reline resins, with Ufi Gel Hard proving to be significantly higher (p<0.001) than Kooliner and Probase Cold, and Kooliner showing disperse component values significantly higher (p=0.028) than Probase Cold. For polar component values, there were no statistical differences (p=0.265) found among reline resins.

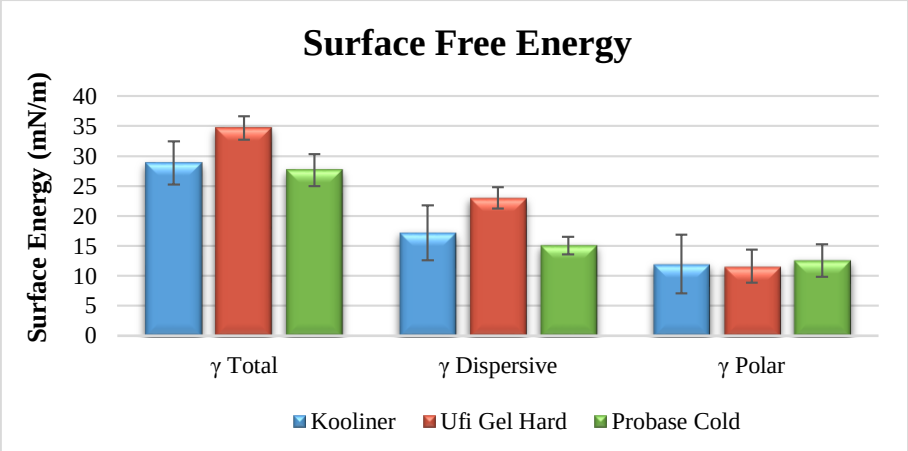

Figure 4.5 – Mean and standard deviation of values of surface free energy (mN/m) by reline resin.

Regarding Kooliner specimens (Figure 4.6), significant differences in total surface free energy (p<0.001) were found, with the Control Group showing significantly lower values than 5% CHX Group (p=0.002) and 7.5% CHX Group (p=0.036). 1% CHX Group demonstrated significant differences (p=0.010) only with 5% CHX Group. The dispersive component also showed statistical differences (p=0.040) between groups. All experimental groups showed no statistical differences among them, except for Control Group that showed a lower dispersive component value than 5% CHX Group (p<0.05). The polar component exhibited statistical differences between groups (p=0.030), with 1% CHX Group showing a lower value than 7.5% CHX Group (p<0.05).

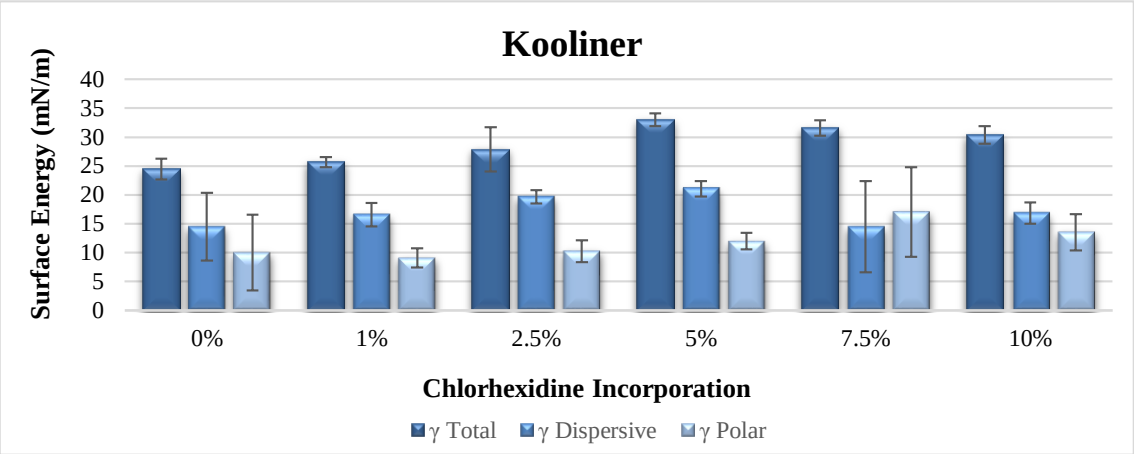

Figure 4.6 – Mean and standard deviation of values of surface free energy (mN/m) of Kooliner.

On Ufi Gel Hard specimens (Figure 4.7), significant differences in total surface free energy (p=0.001) were found. Control Group showed significantly lower values of total surface free energy than 5% CHX Group (p=0.025) and 7.5% CHX Group (p=0.019) and 2.5% CHX Group presented values significantly lower than 7.5% CHX Group (p=0.040). On the contrary, there were no significant differences in the dispersive (p=0.195) and in the polar (p=0.379) components.

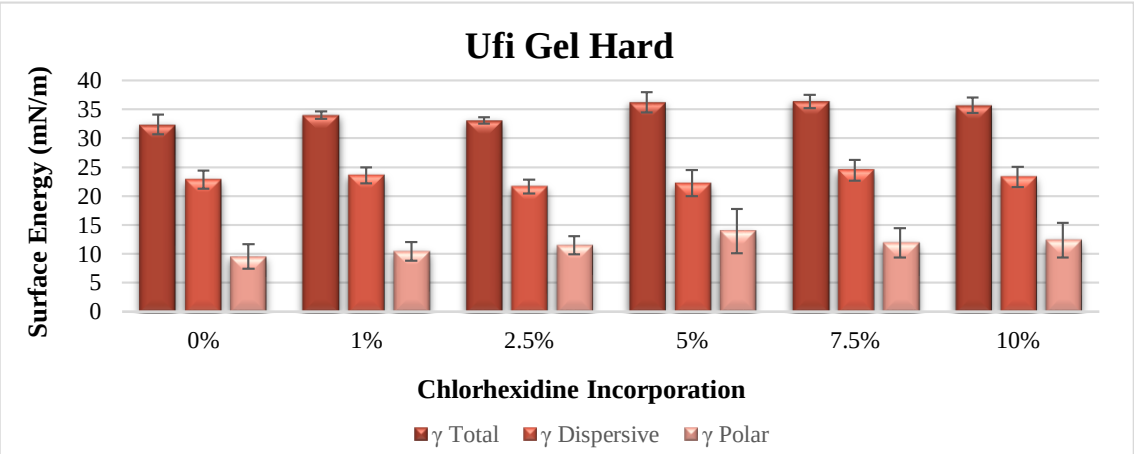

Figure 4.7 – Mean and standard deviation of values of surface free energy (mN/m) of Ufi Gel Hard.

Concerning Probase Cold specimens (Figure 4.8), significant differences in total surface free energy were found (p=0.001). 5% CHX Group demonstrated significant higher values than Control Group (p=0.006) and 1% CHX Group (p=0.036). The dispersive (p=0.295) and polar (p=0.090) components demonstrated no significant differences between groups.

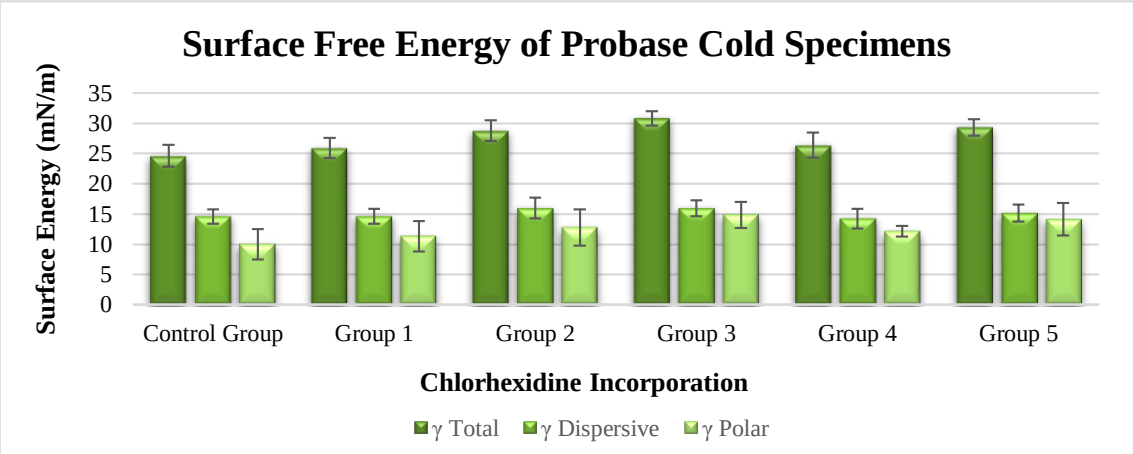

Figure 4.8 – Mean and standard deviation of values of surface free energy (mN/m) of Probase Cold.

5. Discussion

A continuous release delivery system as a therapeutic approach for denture stomatitis using CHX incorporated acrylic resins has been investigated in several microbiological and release studies. All authors concluded that the release of CHX from acrylic resins showed higher rates than other drugs, such as fluconazole, and verified an initial elution at a high rate followed by a slower and steadier diffusion, up to 28 days (Hiraishi, *et al.*, 2008; Amin, *et al.*, 2009; Redding, *et al.*, 2009; Ryalat, *et al.*, 2011; Salim, *et al.*, 2012b; Salim, *et al.*, 2013a; Salim, *et al.*, 2013b; Bertolini, *et al.*, 2014).

Since the incorporation of antimicrobial agents into polymeric materials may affect their properties, their evaluation is particularly important. However, only two studies were found assessing physical properties of acrylic resins with chlorhexidine incorporated (Alcântara, *et al.*, 2012; Salim, *et al.*, 2012a).

The present study evaluated the influence of CHX incorporation on the shear bond strength and surface free energy of three acrylic reline resins (Kooliner, Ufi Gel Hard and Probase Cold). Moreover, since the three resins have different compositions, it seemed appropriate to also compare these physical properties among them.

The first objective of this work was to assess if the shear bond strength values were affected by the acrylic reline resin used.

Bond testing methods, such as shear and tensile tests, are based on the application of a load in order to generate stress until failure occurs. However, to this date, there isn't a consensus on the most reliable test for evaluating the bond strength between denture base and reline resins, according to the current literature (Mutluay and Ruyter, 2005; Valandro, *et al.*, 2008). The bond test used in this investigation applies a shear load directly to the reline-denture base polymer junction, representing a more critical stress than tensile loading (Neppelenbroek, *et al.*, 2006; Elias and Henriques, 2007). Moreover, this test is simpler and specimen preparation is easier, it simulates the loads that affect the relining materials during function and offers useful information on how a modification of the reline resin may affect its mechanical properties (Placido, *et al.*, 2007; Salim, *et al.*, 2012a). On the other hand, tensile tests present difficulty with specimen alignment and a tendency for heterogeneous stress distribution at the adhesive interface (Valandro, *et al.*, 2008).

The results in this study demonstrated that there were statistical differences between acrylic reline resins in shear bond strength values, with Kooliner showing significantly lower values than the two other resins. No differences between Ufi Gel Hard and Probase Cold were presented, however the latter exhibited the highest bond strengths values (40.21 ± 2.52 MPa), on the control group.

Ahmad *et al*. (2009) formulated that when reline materials chemically similar to the denture base are used for relining, the bond strength is higher. This is due to an easier diffusion and penetration of PMMA reline monomers into the similar denture base resin, forming an inter-penetrating polymer network (Takahashi and Chai, 2001a). Probase Cold's higher shear bond values corroborate Ahmad *et al.* (2009) hypotheses, since Probase Cold composition (PMMA/MMA) is identical to Probase Hot, the acrylic resin from the denture base.

On the contrary, Kooliner's lower values may be explained by the composition of its monomer: IBMA is a high molecular weight monomer, therefore its low ability to dissolve the PMMA denture base resin surface leads to a less effective penetration of the reline resin into the denture base (Arima, *et al.*, 1996; Ahmad, *et al.*, 2009). Also, Johnson and Jones (1994) stated that reline resins with increasing concentration of ethyl and butylmethacrylate, as Kooliner, resulted in a linear decrease in the mechanical properties measured.

The absence of differences between Probase Cold and Ufi Gel Hard may be explained by the pronounced standard deviation of the former, owing to a proportional decrease of bond strength values with the increase of CHX concentration incorporated.

At this point, it may be conclude that the first hypothesis of this study could be rejected, since Kooliner showed lower shear bond strength values to denture base resin than the other resins.

Another objective of this study was to evaluate the effect of the incorporation of different CHX concentrations on the shear bond strength of the same acrylic reline resins.

Kooliner and Ufi Gel Hard specimens, showed no differences on shear bond values between groups. From this point of view, we may assume that the CHX incorporation didn't affect the shear bond values on these resins' specimens.

On the contrary, Probase Cold presented significant differences among groups. The Control Group showed the highest shear bond values (40.21±2.52 MPa), decreasing from there in a linear proportion with the concentration of CHX incorporated. In other

words, the higher the concentration of CHX incorporated, the lower were the shear bond values between denture base and Probase Cold reline resin.

Although CHX is soluble in water, it is insoluble in monomers. Thus, CHX could not interfere with the polymerization process of these materials, but its physical presence within the polymer matrix might introduce more spaces and less homogeneity in the polymerized materials (Alcântara, *et al.*, 2012; Salim, *et al.*, 2012a). This fact supports the results obtained with Probase Cold specimens, where the incorporation of CHX weakened the bond strength between the reline resin and the denture.

Alcântara *et al*. (2012) analysed bond values between a denture base and a reline resin with 5% or 10% w/w of CHX incorporated among other antimicrobials, concluding that the incorporation of CHX didn't affect the shear bond values, as the groups didn't show any differences between them. On the contrary, Salim *et al.* (2012) found statistical differences between the control and experimental groups, with the 10% w/w of CHX incorporated specimens exhibiting lower values than the control group. These differences in outcomes among the two studies and the present work may be related with dissimilarities in the acrylic resins selected by each author, since the type of denture base polymer and the type of relining material may affect the bonding properties of different hard relining materials (Mutluay and Ruyter, 2005). Additionally, there are differences in protocol: while the quoted studies prepared the denture base resin for bonding after 24h and 48h immersion in distilled water, in the present study the denture base specimens were submitted to an aging process by thermocycling. In this *in vitro* process, specimens were subjected to temperature extremes (5 and 55 ±2 °C) with a dwell time of 30 seconds and a transfer time of 5 seconds in an attempt to simulate conditions of the oral cavity (Neppelenbroek, *et al.*, 2006). The literature states that 5 000 cycles of cyclic thermal stressing correspond to 6 months of intraoral conditions (Giampaolo, *et al*., 2011), so the 2 500 cycles protocol used in this work simulates a 3-month period of intraoral conditions, considered the minimal interval for a denture evaluation and possible need for base replacement by a relining procedure. Furthermore, the present study compared five different concentrations of the same antimicrobial, whereas the other two studies compared only one or two concentrations to a control group, resorting to other testing machines and bond tests.

Adequate bonding between denture base resin and reline material is essential, since a failure can harbour bacteria, promote staining and cause complete delamination of the relining material. A weak bond will also decrease the strength of the denture and

cause fractures (Takahashi and Chai, 2001a; Pinto, *et al.*, 2004; Mutluay and Ruyter, 2005; Neppelenbroek, *et al.*, 2006; Bettencourt, *et al.*, 2010; Alcântara, *et al.*, 2012).

In this study, the majority (98.33%) of failure mode in Kooliner specimens was adhesive, in agreement with previous studies (Leles, *et al.*, 2001; Takahashi and Chai, 2001b; Ahmad, *et al.*, 2009; Salim, *et al.*, 2012a). An adhesive failure mode may indicate that the bond strength between the reline resin and the denture base is weaker than the reline material strength, which is an advantage if the objective is a temporary lining in practice (Leles, *et al.*, 2001; Salim, *et al.*, 2012a).

Concerning Ufi Gel Hard specimens, the failure mode most obtained was mixed, as Neppelenbroek *et al*. (2006) found in their study. Additionally, the adhesive failures were found in the Control Group and 1% CHX Group, which demonstrated the lower values in shear bond strength.

Probase Cold, which shear bond values decrease with the increase of CHX incorporation, showed a failure pattern in accordance. In other words, the groups with the lower CHX concentration and higher shear bond values obtained a mixed mode, confirming a strong bond between denture base and reline resin polymers; as the concentration of CHX incorporation increased, the percentage of mixed failures decreased and the adhesive failures outnumbered the former.

At this point, it may be conclude that the second hypothesis of this study could be rejected, since Probase Cold presented significantly different shear bond strength values among groups with the incorporation of CHX.

The third objective of the present work was to estimate if there were differences in surface free energy values among the acrylic reline resins.

The determination of surface energy and its dispersive and polar components is based on the method proposed by Wu (1971), which states that if a contact angle (i. e. the interaction between a solid and a liquid surface at the interface) is measured against two liquids of known surface tension and polarity (water and 1,2-propanediol in the present study), then it is possible to estimate the surface energy and polarity of the solid (Zissis, *et al.*, 2001; Bettencourt, *et al.*, 2002).

The impact of surface properties, such as surface free energy, on bacterial adhesion to solid surfaces has not been clarified. Some researchers observed no relation (Hahnel, *et al.*, 2012; da Silva, *et al.*, 2014), whereas other authors concluded that there is an association between the surface free energy values and the *C. albicans* adherence

and proliferation (Minagi, *et al.*, 1985; Moura, *et al.*, 2006; AL-Dwairi, *et al.*, 2012; Koch, *et al.*, 2013). Koch *et al*. (2013) observed that the highest *C. albicans* levels were observed in the substrata with the highest polar contribution to surface free energy and AL-Dwairi *et al*. (2012) stated that the higher the surface free energy, the higher will be the adhesion of microorganisms and alternatively, the more hydrophobic the surface, the less cell adherence is expected.

However, other factors should also be considered, such as cell surface factors, diet, salivary composition and secretion rates, and antibody titers, which are all controlling factors in plaque formation and could therefore influence yeast attachment. This fact might explain why recent studies have failed to show a direct correlation between surface free energy values and the adhesion of *Candida* species (AL-Dwairi, *et al*., 2012).

In the present study, the three resins demonstrated differences in the values of total surface free energy (the sum between its two components) and dispersive (or apolar) component. Ufi Gel Hard demonstrated higher values than the other resins of total surface free energy (34.64±1.70 mN/m), through the increase of its dispersive component. On this basis, it can be deduced that this reline resin has the tendency to be more apolar than Kooliner and Probase Cold, which could translate to a hydrophobicity. Additionally, Kooliner presented significant higher dispersive component values (17.19±2.68 mN/m) than Probase Cold (15.09±0.74 mN/m). Thus, we might say Probase Cold seems to be the most hydrophilic material. The polar component showed no differences between them.

Besides, surface free energy also indicates the ease with which saliva spreads over a surface (Moura, *et al.*, 2006). Accordingly, a higher value of surface free energy corresponds to higher wettability, enhancing the lubricating effect of saliva and thereby promoting denture retention and patient comfort (Zissis, *et al.*, 2001). With this in mind, we might say that Ufi Gel Hard represents the reline resin that could provide the highest retention levels.

At this point, it may be conclude that the null hypothesis of the third objective could be rejected, as Ufi Gel Hard revealed higher surface free energy values than the Kooliner and Probase Cold.

The fourth objective of the present work was to assess if values of surface free energy differ among different concentrations of CHX incorporated.

In the present study, Kooliner demonstrated differences among groups in total surface free energy and in its dispersive and polar components, with 5% CHX Group (by dispersive component) and 7.5% CHX Group (by polar component) showing higher values than the other groups. However, there wasn't found a relation between the incorporation of CHX and the surface properties. This situation can possibly be explained by the difficulty in achieve a total homogenization of the CHX powder in the acrylic reline resin powder, leading to an irregular distribution of particles within the material (Alcântara, *et al.*, 2012).

The technique to determine the surface free energy in this study is an indirect method, since it measures the contact angle by immersing each specimen 4mm into the test liquids (water and 1,2-propanediol, as stated before). Accordingly, the results could beneficiate from an increase of the area submitted to test or from a higher number of times each sample would be submitted to the test.

Regarding Ufi Gel Hard specimens, significant differences in total surface free energy were found between groups, with the groups with higher concentrations of CHX incorporated (5%, 7.5% and 10% w/w) showing the higher values, by increase of the polar component. This means that the incorporation of CHX slightly increases the Ufi Gel Hard's tendency to become polar and, consequently, to become more hydrophilic. There were no significant differences among groups in the dispersive and polar components.

For Probase Cold specimens, there were statistical differences between groups concerning total surface free energy values, with 5% CHX Group obtaining the highest values (30.84±1.20 mN/m). Nonetheless, no significant differences were found in the dispersive and polar components among groups. These results are in accordance with those obtained by Sousa (2014).

Although the total surface free energy is different among groups in Ufi Gel Hard and Probase Cold specimens, the absence of differences in the dispersive and polar components means that there is a balance accomplished by the two components.

To summarize, Kooliner results didn't allow a correlation between the incorporation of CHX and the surface properties; Ufi Gel Hard seems to have the tendency to become polar and, consequently, more hydrophilic with the incorporation of CHX; Probase Cold showed differences only on 5% CHX Group, with higher total surface free energy values, indicating a slightly tendency to become more polar, or hydrophilic.

At this point, it may be conclude that the hypothesis of the fourth objective could be rejected, since all reline resins demonstrate differences in surface free energy values among different concentrations of CHX incorporated.

Several researches investigated the feasibility of using a drug delivery system by incorporation of antimicrobial agents, into denture base or reline resins, in order to reduce the prevalence of denture stomatitis (Amin, *et al.*, 2009; Alcântara, *et al.*, 2012). These studies have been suggesting that the impregnation has potential for prevention microbial adherence, specifically *C. albicans*, prolong the efficiency of oral treatment and decrease the side effects (Amin, *et al.*, 2009; Salim, *et al.*, 2012b; Salim, *et al.*, 2013a).

However, physical properties of the resin seem to be affected by the CHX incorporation, in some concentration levels. The incorporation of chlorhexidine has influenced only shear bond strength of Probase Cold specimens with higher concentrations of chlorhexidine, suggesting that the relining procedure can be carried out with Kooliner and Ufi Gel Hard with any CHX concentration, and with Probase Cold until a CHX concentration of 2.5% w/w. Further studies are required to clarify the extension of the influence, resorting to new methods, such as micro-tensile tests for bonding properties. The surface free energy of the three acrylic reline resins is affected by the incorporation of chlorhexidine, however the effect of this property on *C. albicans* adherence continues unclear, requiring microbiological assays.

Since the roughness of the resin's surface may encourage the colonization by oral microorganisms (Bertolini, *et al.*, 2014), it would be interesting to development a study which also include this parameter for evaluation.

6. Conclusions

Within the limitations of this study, the main conclusions are:

- The shear bond strength to the denture base resin is affected by the acrylic reline resin used, with Kooliner presenting the lowest values of shear bond strength.

- The CHX incorporation affects the shear bond strength only between Probase Cold and the denture base. Bond strength seemed to be maintained in specimens of Kooliner, Ufi Gel Hard and Probase Cold until a CHX concentration of 2.5% w/w.

- The surface free energy values are different between acrylic reline resins. As Ufi Gel Hard seemed to be more apolar than the other resins, Probase Cold appeared to be more polar, or hydrophilic.

- The CHX incorporation alters the surface free energy values of all acrylic reline resins. Ufi Gel Hard and Probase Cold showed a tendency to become more polar with the incorporation of CHX, however, Kooliner didn't obtain a clear relation between surface free energy values and surface properties.

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Appendices

Appendix 1 – Tables

Table 1 – Contact angle data by reline resin.

Material	CHX Incorporated	Contact Angle (°)					
		Water			1,2-Propanediol		
		M±SD	Min	Max	M±SD	Min	Max
Kooliner	Control - 0%	96.09±10.07	91.29	110.74	64.17±2.18	62.35	67.40
	1%	94.12±2.25	91.95	95.77	54.07±3.57	49.70	58.54
	2.5%	88.73±3.62	84.61	93.34	42.38±2.42	40.42	46.37
	5%	83.82±2.51	81.33	87.32	33.96±3.40	30.36	38.87
	7.5%	83.06±1.12	81.86	84.67	53.19±25.69	34.44	97.80
	10%	84.44±5.21	80.92	93.59	46.20±2.13	42.77	48.24
		88.38±5.61			49.00±10.50		
Ufi Gel Hard	Control - 0%	87.99±4.13	83.35	92.34	33.75±4.72	27.85	41.03
	1%	85.41±2.81	82.78	89.10	28.55±2.01	25.63	31.23
	2.5%	84.54±2.46	81.11	86.95	33.12±1.74	31.31	35.29
	5%	79.12±5.96	71.11	84.36	26.04±2.47	22.67	29.54
	7.5%	81.73±4.35	75.60	87.19	20.69±3.06	18.10	25.93
	10%	81.61±5.13	75.19	88.45	25.04±2.67	21.29	27.81
		83.40±3.19			27.87±5.01		
Probase Cold	Control - 0%	94.03±4.80	87.13	99.80	58.63±2.57	54.85	61.04
	1%	91.12±4.47	87.27	96.43	56.51±2.26	54.72	60.32
	2.5%	86.76±4.78	81.10	92.32	50.10±2.81	46.98	53.63
	5%	82.42±3.96	77.43	85.29	47.06±2.39	42.94	48.82
	7.5%	89.64±3.00	86.52	94.58	56.11±5.74	52.54	66.01
	10%	84.74±3.93	79.25	89.50	50.73±1.55	49.00	52.97
		88.12±4.29			53.19±4.52		

M = Mean, SD = Standard deviation, Min = Minimum, Max = Maximum.

Table 2 – Surface Free Energy data by reline resin.

Material	CHX Incorporated		Surface Free Energy (γ) (mN/m)		
			γ Total	γ Dispersive	γ Polar
Kooliner	Control - 0%	M±SD	24.48±1.82	14.48±5.85	10.06±6.56
		Min	22.0	9.3	0.8
		Max	27.1	24.1	17.8
	1%	M±SD	25.68±0.91	16.60±2.04	9.08±1.67
		Min	24.4	14.9	7.3
		Max	26.6	19.3	11.2
	2.5%	M±SD	27.92±3.83	19.64±1.16	10.26±1.90
		Min	21.3	18.9	8.2
		Max	30.4	21.6	12.4
	5%	M±SD	33.04±1.10	21.06±1.30	12.00±1.44
		Min	31.6	19.6	9.9
		Max	34.4	22.4	13.6
	7.5%	M±SD	31.56±1.34	14.50±7.87	17.06±7.77
		Min	29.6	1.0	12.1
		Max	32.9	20.8	30.8
	10%	M±SD	30.40±1.50	16.88±1.86	13.52±3.13
		Min	28.0	15.7	8.0
		Max	31.8	20.0	15.7
			28.85±3.39	17.19±2.68	12.00±2.94
Ufi Gel Hard	Control - 0%	M±SD	32.38±1.69	22.84±1.54	9.54±2.12
		Min	29.7	20.9	7.3
		Max	34.3	24.9	12.3
	1%	M±SD	34.04±0.65	23.58±1.34	10.44±1.61
		Min	32.9	22.4	8.6
		Max	34.4	25.6	12.0
	2.5%	M±SD	33.10±0.58	21.64±1.23	11.48±1.54
		Min	32.2	19.7	9.9
		Max	33.7	23.1	13.8
	5%	M±SD	36.24±1.73	22.22±2.26	13.98±3.83
		Min	34.8	19.7	10.7
		Max	38.9	24.3	19.0
	7.5%	M±SD	36.38±1.18	24.46±1.81	11.92±2.55
		Min	34.7	22.3	8.6
		Max	37.9	27.3	15.6

	10%	**M±SD**	35.68±1.33	23.30±1.78	12.38±3.01
		Min	33.9	20.5	8.6
		Max	37.0	25.3	16.5
			34.64±1.70	23.01±1.00	11.62±1.55
Probase Cold	**Control - 0%**	**M±SD**	24.62±1.79	14.60±1.21	10.02±2.54
		Min	23.1	13.2	6.7
		Max	27.5	16.5	13.4
	1%	**M±SD**	25.94±1.67	14.60±1.24	11.34±2.49
		Min	23.7	13.8	7.9
		Max	27.5	16.8	13.3
	2.5%	**M±SD**	28.78±1.72	15.98±1.72	12.78±2.99
		Min	26.4	13.8	9.7
		Max	31.1	18.4	16.0
	5%	**M±SD**	30.84±1.20	15.96±1.30	14.88±2.18
		Min	29.6	14.3	13.1
		Max	32.7	17.8	18.4
	7.5%	**M±SD**	26.40±2.08	14.24±1.61	12.18±0.89
		Min	22.8	11.6	11.3
		Max	28.1	15.6	13.4
	10%	**M±SD**	29.32±1.39	15.18±1.41	14.14±2.71
		Min	27.9	13.2	10.9
		Max	31.3	17.1	18.1
			27.65±2.36	15.09±0.74	12.56±1.79

M = Mean, SD = Standard deviation, Min = Minimum, Max = Maximum.

Appendix 2 – Figures

Figure 1 – Probase Hot (PH).

Figure 2 – Kooliner (K).

Figure 3 – Ufi Gel Hard (U).

Figure 4 – Probase Cold (PC).

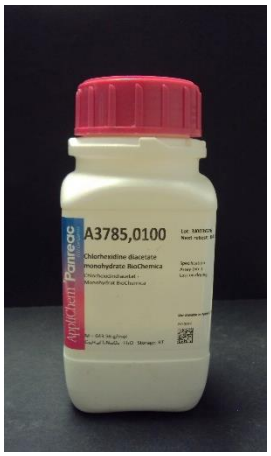

Figure 5 – Chlorhexidine diacetate monohydrate (CHX).

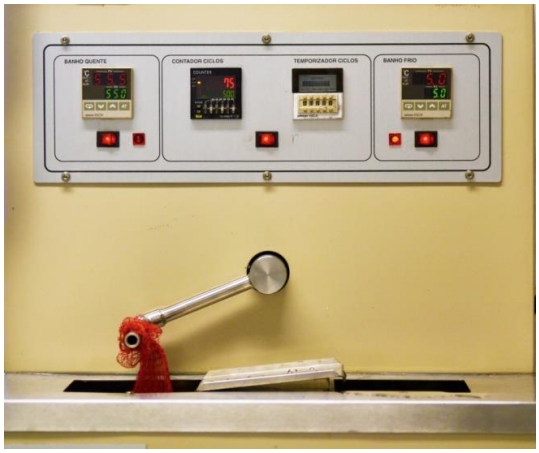

Figure 6 – Themocycling equipment.

Figure 7 – Ivomat pressure device.

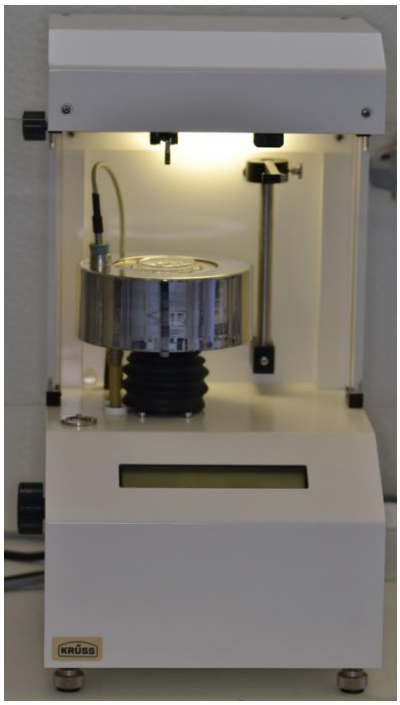

Figure 8 – Processor Tensiometer K12: Equipment used in Wilhelmy Plaque technique.

Figure 9 – Processor Tensiometer K12: Equipment used in Wilhelmy Plaque technique.

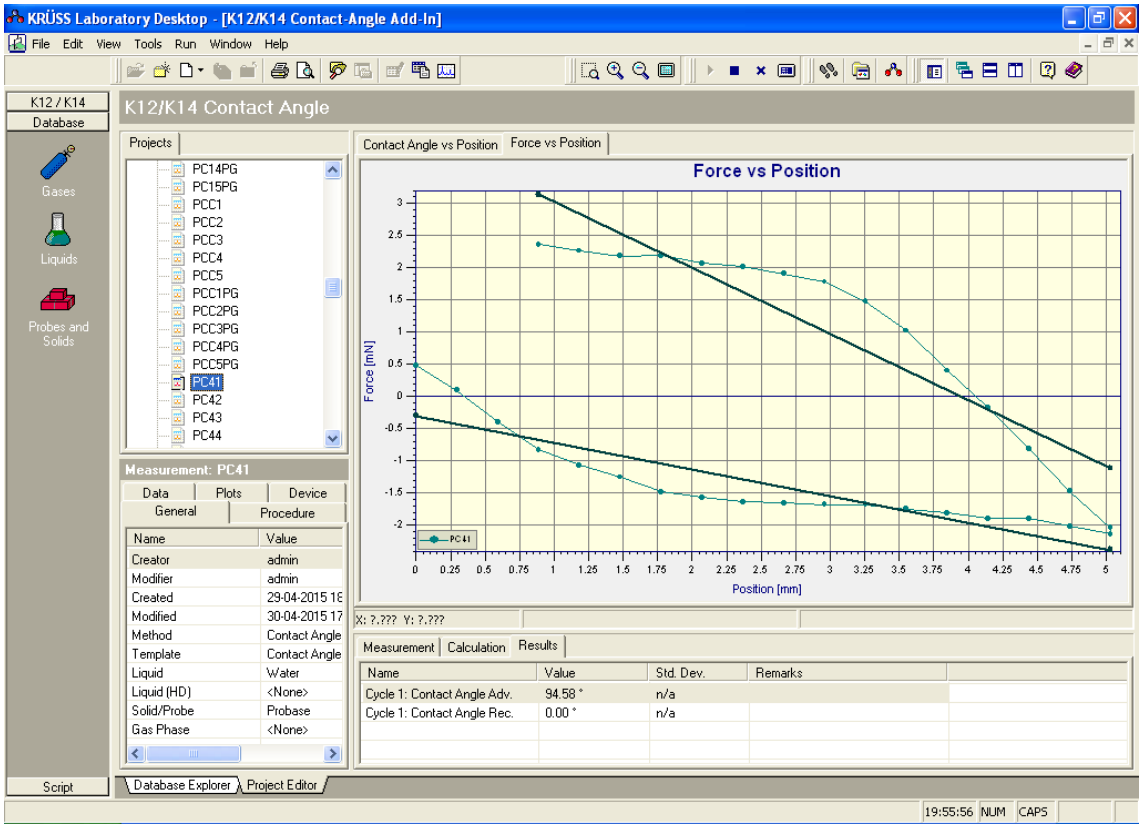

Figure 10 – KRUSS-software program: contact angle measuring system K121 (version 2.049).

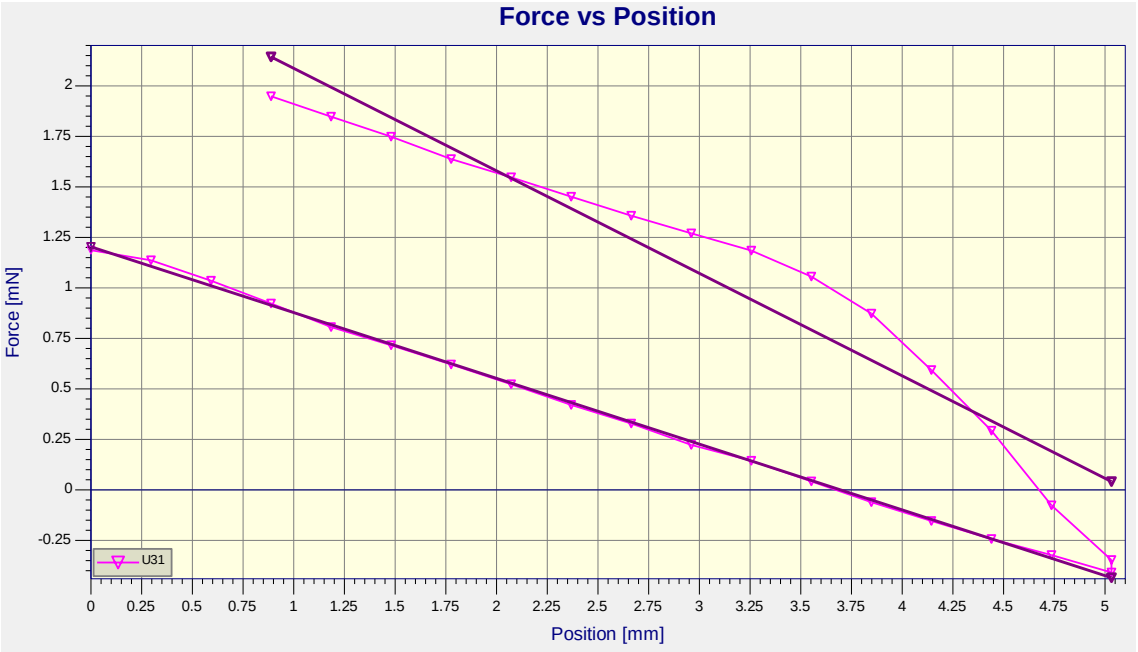

Figure 11 – One example of graphical obtained for determination of the contact angle of an Ufi Gel Hard specimen.

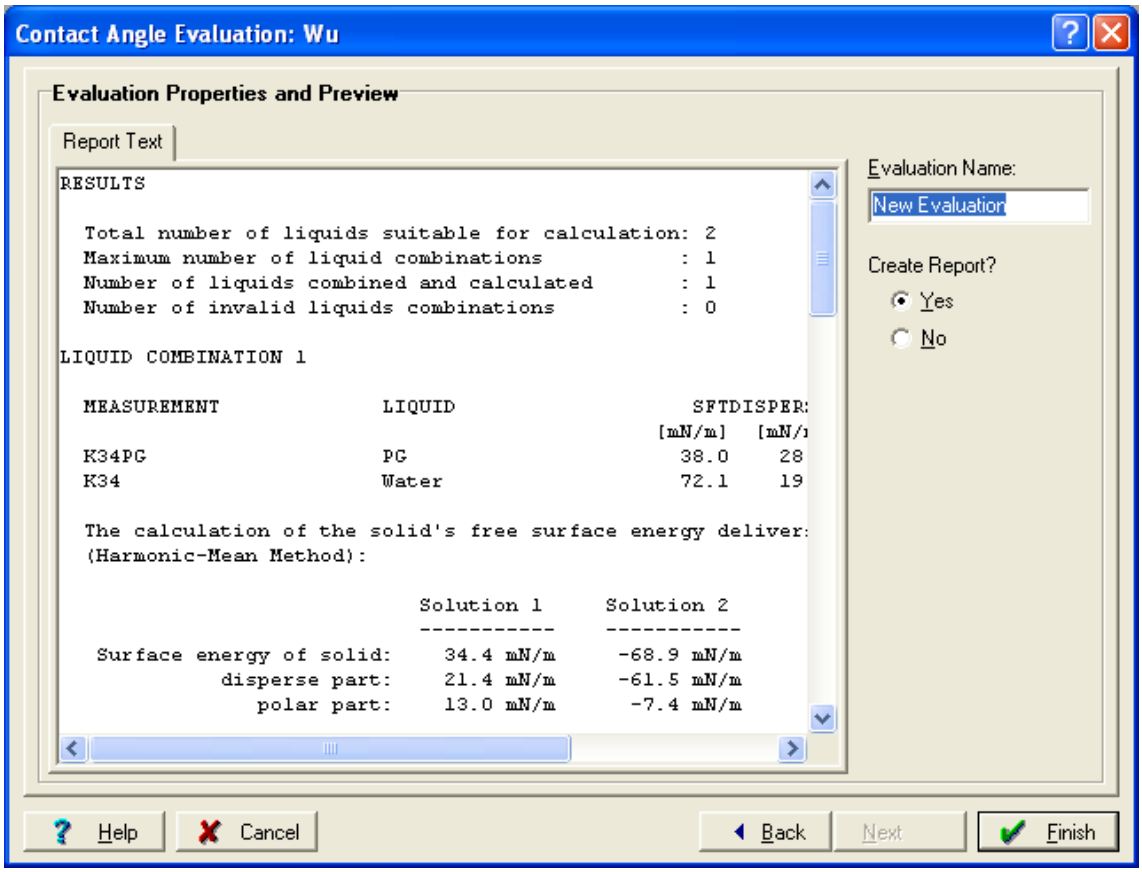

Figure 12 – One example of determination of the surface free energy of a Kooliner specimen.

Appendix 3 – List of Tables

Appendix 4 – List of Figures

Appendix 5 – List of Abbreviations

1,6-HDMA	1,6-hexanedioldimethacrylate.
CHX	Chlorhexidine diacetate monohydrate
CON	Conditioner
HEMA	2-hydroxyethylmethacrylate
IBMA	Isobutylmethacrylate
K	Kooliner
L	Liquid
M	Mean
Max	Maximum
Min	Minimum
MMA	Methylmethacrylate
MPa	Megapascal
P	Powder
PC	Probase Cold
PEMA	Polyethylmethacrylate
PH	Probase Hot
PMMA	Polymethylmethacrylate
SD	Standard deviation
SF	Separating fluid
U	Ufi Gel Hard
γ	Surface free energy
γ^d	Dispersive component of surface free energy
γ^p	Polar component of surface free energy

Appendix 6 - Experimental Data

1. Shear bond strength

1.1. Kooliner

Specimen	CHX Incorporation	Shear Bond Stregth (MPa)	Failure Mode	Specimen	CHX Incorporation	Shear Bond Stregth (MPa)	Failure Mode
KC1	Control 0%	12.62	adhesive	K31	5%	23.16	adhesive
KC2		15.72	adhesive	K32		20.80	adhesive
KC3		15.16	adhesive	K33		15.85	adhesive
KC4		7.659	adhesive	K34		15.97	adhesive
KC5		11.71	adhesive	K35		12.84	adhesive
KC6		20.31	adhesive	K36		13.17	adhesive
KC7		11.54	adhesive	K37		13.40	adhesive
KC8		12.72	adhesive	K38		17.98	adhesive
KC9		13.74	adhesive	K39		22.64	adhesive
KC10		10.20	adhesive	K310		19.30	adhesive
K11	1%	18.04	adhesive	K41	7.5%	20.69	adhesive
K12		17.83	adhesive	K42		16.65	adhesive
K13		18.31	adhesive	K43		16.39	adhesive
K14		22.73	adhesive	K44		15.73	adhesive
K15		17.24	adhesive	K45		18.25	adhesive
K16		10.77	adhesive	K46		23.45	adhesive
K17		17.92	adhesive	K47		15.40	adhesive
K18		19.52	adhesive	K48		18.41	adhesive
K19		8.931	mixed	K49		11.36	adhesive
K110		10.12	adhesive	K410		19.99	adhesive
K21	2.5%	16.10	adhesive	K51	10%	15.95	adhesive
K22		17.71	adhesive	K52		15.89	adhesive
K23		16.31	adhesive	K53		16.96	adhesive
K24		20.35	adhesive	K54		18.44	adhesive
K25		17.80	adhesive	K55		19.45	adhesive
K26		16.33	adhesive	K56		19.43	adhesive
K27		16.59	adhesive	K57		15.97	adhesive
K28		22.67	adhesive	K58		16.89	adhesive
K29		29.41	adhesive	K59		22.58	adhesive
K210		14.63	adhesive	K510		13.69	adhesive

1.2. Ufi Gel Hard

Specimen	CHX Incorporation	Shear Bond Stregth (MPa)	Failure Mode	Specimen	CHX Incorporation	Shear Bond Stregth (MPa)	Failure Mode
UC1	Control 0%	23.21	adhesive	U31	5%	28.72	mixed
UC2		24.39	adhesive	U32		28.58	mixed
UC3		23.75	adhesive	U33		22.91	mixed
UC4		23.02	mixed	U34		26.33	mixed
UC5		26.40	adhesive	U35		30.99	mixed
UC6		21.47	adhesive	U36		31.13	mixed
UC7		25.07	adhesive	U37		29.01	mixed
UC8		23.99	adhesive	U38		29.35	mixed
UC9		28.41	adhesive	U39		26.16	mixed
UC10		25.36	adhesive	U310		27.77	mixed
U11	1%	21.71	adhesive	U41	7.5%	22.33	mixed
U12		20.93	adhesive	U42		23.08	adhesive
U13		27.33	adhesive	U43		23.81	mixed
U14		24.87	adhesive	U44		29.68	mixed
U15		22.05	adhesive	U45		24.03	mixed
U16		27.55	adhesive	U46		29.83	mixed
U17		22.41	mixed	U47		25.32	mixed
U18		26.14	adhesive	U48		28.16	adhesive
U19		18.12	adhesive	U49		27.46	mixed
U110		24.30	adhesive	U410		28.87	mixed
U21	2.5%	26.62	mixed	U51	10%	22.59	mixed
U22		26.58	mixed	U52		16.84	mixed
U23		26.63	mixed	U53		20.87	mixed
U24		31.36	mixed	U54		25.34	mixed
U25		28.48	mixed	U55		30.52	adhesive
U26		30.76	mixed	U56		21.76	mixed
U27		26.52	mixed	U57		27.90	adhesive
U28		22.04	mixed	U58		27.84	mixed
U29		32.38	mixed	U59		25.34	adhesive
U210		25.56	mixed	U510		25.19	mixed

1.3. Probase Cold

Specimen	CHX Incorporation	Shear Bond Stregth (MPa)	Failure Mode	Specimen	CHX Incorporation	Shear Bond Stregth (MPa)	Failure Mode
PCC1	Control 0%	40.16	mixed	PC31	5%	25.95	mixed
PCC2		38.95	adhesive	PC32		25.98	adhesive
PCC3		43.15	mixed	PC33		26.71	mixed
PCC4		41.34	mixed	PC34		26.38	adhesive
PCC5		40.01	mixed	PC35		25.63	mixed
PCC6		42.01	mixed	PC36		20.86	mixed
PCC7		40.95	adhesive	PC37		25.67	mixed
PCC8		33.81	mixed	PC38		18.12	adhesive
PCC9		41.10	mixed	PC39		15.75	adhesive
PCC10		40.60	mixed	PC310		24.33	adhesive
PC11	1%	38.94	mixed	PC41	7.5%	16.35	adhesive
PC12		34.70	mixed	PC42		19.61	adhesive
PC13		31.78	mixed	PC43		13.55	adhesive
PC14		37.10	mixed	PC44		17.43	adhesive
PC15		32.64	mixed	PC45		17.77	adhesive
PC16		36.69	mixed	PC46		17.08	adhesive
PC17		36.54	mixed	PC47		19.23	adhesive
PC18		36.55	mixed	PC48		11.70	adhesive
PC19		40.25	mixed	PC49		14.25	adhesive
PC110		30.74	adhesive	PC410		13.33	mixed
PC21	2.5%	27.16	mixed	PC51	10%	14.80	adhesive
PC22		31.41	adhesive	PC52		15.86	adhesive
PC23		31.64	mixed	PC53		18.17	adhesive
PC24		32.31	mixed	PC54		15.25	adhesive
PC25		31.76	mixed	PC55		16.08	adhesive
PC26		26.66	mixed	PC56		18.21	adhesive
PC27		31.64	mixed	PC57		17.97	adhesive
PC28		27.75	adhesive	PC58		10.74	adhesive
PC29		27.07	adhesive	PC59		9.06	adhesive
PC210		27.87	adhesive	PC510		17.93	adhesive

2. Surface free energy

2.1.Kooliner

Specimen	Width (mm)	Height (mm)	Thickness (mm)	Advance Contact Angle (°)		Surface Free Energy (γ) (mN/m)		
				Water	1,2-Propanediol	γ Total	γ Dispersive	γ Polar
KC1	25.10	17.00	1.00	91.29	65.40	24.2	11.0	13.2
KC2	25.60	17.40	1.24	92.14	62.22	24.3	12.4	12.0
KC3	25.40	16.60	1.20	84.93	67.40	27.1	9.3	17.8
KC4	25.20	16.70	1.20	101.34	62.35	22.0	15.6	6.5
KC5	25.30	16.03	1.20	110.74	63.27	24.8	24.1	0.8
K11	25.00	16.80	1.02	96.98	56.31	24.4	16.6	7.8
K12	25.00	16.38	1.00	95.77	49.70	26.6	19.3	7.3
K13	25.20	16.32	1.08	93.96	51.47	26.3	17.6	8.7
K14	25.02	15.82	1.08	91.95	54.32	26.0	15.6	10.4
K15	25.00	15.98	1.00	91.96	58.54	25.1	13.9	11.2
K21	25.74	16.42	1.15	93.34	46.37	27.9	19.8	8.2
K22	25.51	16.46	1.25	91.61	40.56	29.9	21.6	8.3
K23	25.68	16.67	1.22	84.61	40.42	21.3	18.9	12.4
K24	25.49	15.67	1.32	87.31	42.63	30.1	18.9	11.1
K25	25.55	15.61	1.25	86.79	41.94	30.4	19.0	11.3
K31	25.29	16.17	1.12	87.32	34.02	32.3	22.4	9.9
K32	25.22	16.57	1.08	81.45	35.29	33.4	19.8	13.6
K33	25.37	16.17	1.09	84.80	38.87	31.6	19.6	12.1
K34	25.22	16.39	1.07	81.33	30.36	34.4	21.4	13.0
K35	25.18	16.17	1.06	84.18	31.26	33.5	22.1	11.4
K41	25.69	16.44	1.35	83.69	34.44	32.9	20.8	12.1
K42	25.42	16.56	1.38	82.59	97.80	31.8	1.0	30.8
K43	25.42	16.37	1.24	82.48	37.47	32.6	19.3	13.3
K44	25.37	16.62	1.33	84.67	48.79	29.6	15.8	13.8
K45	25.47	16.38	1.22	81.86	47.47	30.9	15.6	15.3
K51	25.34	16.71	1.10	93.59	46.04	28.0	20.0	8.0
K52	25.22	16.01	1.06	83.54	48.24	30.1	15.7	14.4
K53	25.08	16.62	1.13	81.64	42.77	31.8	17.2	14.6
K54	25.25	16.00	1.09	80.92	46.32	31.5	15.8	15.7
K55	25.27	15.83	1.04	82.50	47.65	30.6	15.7	14.9

2.2.Ufi Gel Hard

Specimen	Width (mm)	Height (mm)	Thickness (mm)	Advance Contact Angle (°)		Surface Free Energy (γ) (mN/m)		
				Water	1,2-Propanediol	γ Total	γ Dispersive	γ Polar
UC1	25.18	16.66	1.14	83.35	33.90	33.1	20.9	12.3
UC2	25.20	15.62	1.12	92.34	41.03	29.7	21.8	7.9
UC3	24.93	16.70	1.23	91.64	33.23	32.3	24.9	7.3
UC4	25.12	16.51	1.27	88.43	32.74	32.5	23.4	9.1
UC5	24.97	16.75	1.19	84.20	27.85	34.3	23.2	11.1
U11	25.12	17.00	1.24	87.69	25.63	34.4	25.6	8.8
U12	24.98	16.78	1.19	84.03	29.03	34.1	22.8	11.3
U13	24.63	17.23	1.23	83.45	28.1	34.4	22.8	11.5
U14	24.76	17.77	1.17	89.10	31.23	32.9	24.3	8.6
U15	25.00	16.68	1.24	82.78	28.77	34.4	22.4	12
U21	24.58	16.30	0.93	86.95	31.60	33.00	23.10	9.90
U22	24.78	16.79	1.11	86.82	34.47	32.20	22.00	10.30
U23	24.80	16.52	1.12	81.11	35.29	33.50	19.70	13.80
U24	24.53	16.68	1.06	84.45	32.91	33.10	21.60	11.50
U25	24.80	16.87	1.08	83.37	31.31	33.70	21.80	11.90
U31	24.64	16.54	1.14	71.11	26.32	38.9	19.9	19.0
U32	24.79	15.28	1.10	84.36	25.25	34.8	24.1	10.7

U33	24.99	16.16	1.19	74.39	29.54	37.0	19.7	17.2
U34	24.84	17.10	1.20	82.65	26.44	34.9	23.1	11.8
U35	24.94	16.96	1.32	83.10	22.67	35.6	24.3	11.2
U41	25.32	16.87	1.31	80.84	19.49	36.6	24.3	12.3
U42	25.50	16.96	1.17	75.60	20.57	37.9	22.3	15.6
U43	25.34	16.83	1.21	87.19	19.38	35.9	27.3	8.6
U44	25.25	16.40	1.26	80.71	18.10	36.8	24.5	12.3
U45	25.41	17.19	1.28	84.29	25.93	34.7	23.9	10.8
U51	25.02	16.78	1.03	88.45	27.81	33.9	25.3	8.6
U52	25.11	16.73	1.06	83.20	24.00	35.3	24.0	11.3
U53	24.86	17.45	1.11	83.13	24.72	35.2	23.8	11.4
U54	24.97	17.12	1.09	78.06	21.29	37.0	22.9	14.1
U55	24.92	16.92	1.06	75.19	27.39	37.0	20.5	16.5

2.3.Probase Cold

Specimen	**Width (mm)**	**Height (mm)**	**Thickness (mm)**	**Advance Contact Angle (º)**		**Surface Free Energy (γ) (mN/m)**		
				Water	**1,2-Propanediol**	**γ Total**	**γ Dispersive**	**γ Polar**
PCC1	25.05	16.73	1.06	97.04	61.04	23.10	14.40	8.70
PCC2	25.28	16.05	1.21	93.50	57.34	24.90	14.80	10.00
PCC3	25.67	15.62	1.22	99.80	59.19	23.20	16.50	6.70
PCC4	25.64	16.63	1.25	92.66	60.72	24.40	13.20	11.30
PCC5	25.43	16.39	1.23	87.13	54.85	27.50	14.10	13.40
PC11	25.22	16.35	1.35	96.43	55.43	24.70	16.80	7.90
PC12	25.36	16.05	1.14	95.48	60.32	23.70	14.20	9.50
PC13	25.37	15.10	1.11	88.93	56.76	26.50	13.80	12.70
PC14	24.27	15.88	1.20	87.27	54.72	27.50	14.20	13.30
PC15	25.58	15.44	1.10	87.48	55.32	27.30	14.00	13.30
PC21	24.95	16.29	1.22	90.93	46.98	28.10	18.40	9.70
PC22	24.88	16.23	1.23	92.32	52.45	26.40	16.60	9.80
PC23	24.87	16.11	1.16	81.10	48.43	31.10	15.10	16.00
PC24	25.13	16.21	1.24	85.90	49.02	29.10	16.00	13.00
PC25	24.15	16.56	1.17	83.55	53.63	29.20	13.80	15.40
PC31	25.20	15.83	1.05	81.76	47.81	30.90	15.50	15.40
PC32	25.18	16.86	1.07	83.88	42.94	31.00	17.80	13.20
PC33	25.59	16.20	1.01	83.72	48.51	30.00	15.70	14.30
PC34	24.95	16.04	1.04	85.29	47.23	29.60	16.50	13.10
PC35	25.79	15.85	1.01	77.43	48.82	32.70	14.30	18.40
PC41	24.90	15.97	1.05	94.58	66.01	22.80	11.60	11.30
PC42	24.57	16.00	1.11	88.75	56.22	26.70	14.00	12.70
PC43	24.94	15.68	1.10	89.74	52.74	27.00	15.60	11.40
PC44	25.11	15.89	1.20	86.52	53.02	28.10	14.60	13.40
PC45	25.01	15.89	1.17	88.60	52.54	27.40	15.40	12.10
PC51	25.03	16.56	1.11	82.64	49.82	30.20	14.90	15.20
PC52	25.82	15.90	1.03	86.66	50.37	28.50	15.70	12.80
PC53	25.70	16.65	1.14	79.25	52.97	31.30	13.20	18.10
PC54	24.82	15.51	1.06	89.50	49.00	27.90	17.10	10.90
PC55	25.49	16.41	1.10	85.63	51.51	28.70	15.00	13.70